Workshop
Assaying Potency of Novel Vaccines
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Lot-Release Assays Are Necessary to Help Provide Assurance of the Quality and Consistency of Vaccine Products and to Help Establish Compliance with FDA Approved Manufacturing Process

Lot-Release Assays May Change Over Time and With Stage of Clinical Development

- With new scientific information, the tests may change and new tests may be added

Recent example: Reverse transcriptase assay
Future example: Assay for TSE

- Specific lot-release tests / release criteria may depend on the stage of product development

General Lot-Release Tests for Viral Vaccines

Sterility
Purity
Identity
Potency
Safety

Different Types of Vaccines May Have Specific Requirements for Lot-Release Tests Including Potency Assays

- DNA Vaccines

  e.g., HIV, SARS virus, ebola virus
- Recombinant Protein Vaccines

  e.g., hepatitis B virus
- Inactivated Vaccines

  e.g., hepatitis A virus, influenza virus, IPV, rabies
- Live, Attenuated Viral Vaccines

  e.g., MMR, yellow fever, varicella

Regulatory definitions of Potency - 1

21 CFR §610.10

Tests for potency shall consist of either *in vitro* or *in vivo* tests, or both, which (*sic*) have been specifically designed for each product so as to indicate its potency in a manner adequate to satisfy the interpretation of potency given by the definition in 21 CFR §600.3(s)

Regulatory Definition of Potency - 2

21 CFR §600.3(s)

The word ***potency*** is interpreted to mean the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result

Aim of a Potency Assay

A potency assay should be predictive of immune protection

Commonly Used Vaccine Potency Tests

- Correlation of a laboratory assay or animal immune response to the expected human immunological response in a dose-dependent manner
- Antigen quantitation in the final formulation
- Direct quantitation of replicating immunogen (*e.g*. attenuated microbial agents)

Potency Tests for Live, Viral Vaccines

- *in vitro* titer of virus
- *in vitro* expression of antigen

  Infection of indicator cells

  - product assessed by: Western analysis, ELISA,
    immunostaining of plaques, foci, etc.
    flow cytometry
- *in vivo* immunogenicity of antigen

  Infection of animals (usually rodents)

  - product assessed by: humoral response
    cellular response

Potency Assays: Usefulness of B-cell Epitopes

The inclusion of B-cell epitopes may be advantageous for both *in vitro* and and *in vivo* potency assays:

- For Western blots of transfected or infected cells
- For immunogenicity studies in small animals

Antibody Binding assays:

- Stability/immunogenicity indicating dose range
- Serial dilutions of immune sera are recommended

Antibody functional assays:

- Desirable for advanced trials
- Help to establish correlates of protection

Potency Assays: Vaccine Containing Only T-Cell Epitopes

- **Challenges (I):**
  - Cross-presentation of HLA class-I or class II restricted epitopes following uptake of exogenous peptides must be demonstrated.
  - Intracellular processing and presentation of multi-epitope cassettes or peptides may differ from that of intact proteins expressed by bacterially- or virally- infected cells.

Potency Assays: Vaccines Containing Only T-Cell Epitopes

- **Challenges (II):**
  - Western blots of transfected/infected cells may not be possible for all inserts (if antibody epitopes are not present)
  - Most epitopes recognized by human HLA are not presented by murine MHC molecules
  - Limited number of HLA transgenic mouse are available for *in vivo* testing

In Vitro Tests for Potency

Pros

- Straightforward
- Reproducible
- Quantitative or semi-quantitative
- Often appropriate for multiple antigen vaccines
- Generally inexpensive
- Usually rapid

Cons

- Does not measure intended outcome of vaccine
  i.e., immune response

In vivo Immunogenicity Tests for Potency

Pros

- Measure intended outcome of vaccine
  e.g., humoral response may be similar to humans

Cons

- Level of response required for immunity may not be established
- Not always quantitative
- May not be reproducible even with inbred strains
- T-cell responses (CTL epitopes) may not be similar in absence of human MHC class I (HLA-transgenics may help)

Potency Assays:
Step-Wise Development

Potential *in vitro* assays for development during phase I/II trials of plasmids or viral and bacterial vectors:

- Transcription of the gene inserts in relevant human cells. Q-PCR for maximal quantitation
- Western blots of transfected/infected cells using antibodies against insert gene products. How to improve quantitation?
- Protein expression in cell extracts by quantitative ELISA

Potency Assays: Step-Wise Development

Potential *in vitro* assays for development during phase I/II trials of plasmids or viral vectors: "Immunological Recognition"

- Recognition of class-I or class-II restricted epitopes presented by transfected/infected human cells to antigen-specific T cell lines established from infected individuals or vaccinated animals.
  - What read-out?
  - How to quantitate?

Potency Assays: Step-Wise Development

In preparation for phase III trials:

- Demonstrate direct correlation between the quantitative *in vitro* assay and immunogenicity (*in vivo* or *in vitro)*
- Prepare a reference vaccine product as well as positive and negative controls. Establish statistical plan to identify loss of potency in a given vaccine lot.
- Establish rigorous release criteria.
- Use the validated potency assay in real-time and/or accelerated stability tests.

Potency Assays:
Step-Wise Development

Post licensure:

- Demonstrate direct correlation between the selected potency assay and protection in the pivotal efficacy trials
- Adjust release criteria accordingly.
- Prepare a reference vaccine product as well as positive and negative controls. Establish statistical plan for release of future vaccine lots for marketing.